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FIG. 1

NUCLEIC ACID AND PREDICTED PROTEIN SEQUENCE OF DZ2

NcoI

1	GGCACGAGCAGAATCGAAGATGGCAACAAAATCCAT <u>GGGAGATATCGAGAAATAAGAA</u>	60
	M A T K S M G D I E K I K K	
61	GAAACTAACGTGTTGATCGTCGATGATGATCCACTAAACCTTATAATTCAATGAGAAAGAT	120
	K L N V L I V D D P L N L I I H E K I	
	↓	
	DZ2FL →	
121	CATCAAAGCGATTGGGGTATTTCACAGACAGCGAATAACGGTGAGGAGGCAGTAATCAT	180
	I K A I G G I S Q T A N N G E E A V I I	
181	<u>CCACCGTGACGGGGCTCATCTTGCACCTTATCCTAATGGATAAGAAATGCCCGAGAG</u>	240
	H R D G G S S F D L I L M D K E M P E R	
241	GGATGGTGTGACAACAACTAAGAAGCTAACAGAGAAATGGAAAGTCAATGATGTTGG	300
	D G V S T T K L R E M E V K S M I V G	
301	GGTGAECTCACTGGCTGACAATGAAGAGGAGCCAGGGCTTCATGGAAAGCTGGACTTAA	360
	V T S L A D N E E R R A F M E A G L N	

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361	CCATTGGCAAAACCGTTAACCAAGGACAAGGATCATCCCTCATTAACCAACTCAT	420
	H C L A K P L T K D K I I P L I N Q L M	
	\leftarrow DZ2RL	
421	GGATGCCTTGATGGATATATTTATGGAAACACATAATAACGTCTAAGTGTG	480
	D A *	
481	<u>TATGTATGCATAGATACTTGCATGTGTGTTAGAATTAGGGTTCTTTATCGTCCGT</u>	540
	<i>HindIII</i>	
541	GATATATAATCATGTAAGTGTGCTTAAAGCTTATAAAATATTAAATAAGGGTTCCCT	600
601	CTACC	

The primer sites for DZ2FL and DZ2RL are underlined, as are the recognition sequences for *Nco*I and *Hind*III restriction enzymes. Shown in bold are the conserved amino acid residues required for phosphorylation. The extent of the original PCR product isolated by differential display is shown by ↓.

FIG. 1 CONT'D

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Alignment of the predicted protein sequence of DZ2 with those of
bacterial response regulator proteins.

DZ2	MATKSMGDIE KIKKKLNVL VDDDPPLNLII HEKIIKAIG. GISQTANNGE	50
OMPRMQENYKILV VDDDMRLRAL LERYLTEQGF	.QVRSVANAE
PHOBMARRILV VEDEAPIREM VCFVLEQNGF	.QPVEAEDYD
NTRC	...MQRGIVWV VDDDSSIRWV LERALAGAGL	.TCTTFENGN
SPOOF	..MMNEKILI VDDQYGIRIL LNEVFNKEGY	.QTFOAANGL
CHEYMADKELKFLV VDDFSTMRRV VRNLLKELGF NNVEEAEIGV	
ETRLKVLV MDENGVSRMV TKGLLVHLGC EVTTVSSNEE	
DZ2	EAVIIHRDGG SSFDLILMDK EMPERDGVST TKKLREMEVK SM..IVGVT	100
OMPR	QMDRLLTR.. ESFHLMVLDL MLPGEDGLSI CRRLRSQS.. NPMPIIMVTA	
PHOB	SAVNQLNE.. PWPDLLLWD MLPGGSGIQF IKHLKRESMT RDIPVVMLTA	
NTRC	EVLAALAS.. KTPDVLLSDI RMPGMDGLAL LKQIKQ..RH PMLPVIIMTA	
SPOOF	QALDIVTK.. ERPDVLVLLDM KIPGMGIEI LKRMKV..ID ENIRVIIMTA	
CHEY	DALNKLQA.. GGYGFVISDW NMPNMDGLEL LKTIRADGAM SALPVLMVTA	
ETRCLRVVS HEHKVVFMVDV CMPGVENYQI ALRI..... HXPLLVALS	
DZ2	LADNEEERRA FMEAGLNHCL AKPLTKDKII PLINQLMDA.	150
OMPR	KGEEVDRIVG .LEIGADDIY PKPFPNPRELL ARIRAVLRRQ ANELPGAPS.	
PHOB	RGEEDDRVKG .LETGADDIY TKPFSPKELV ARIKAVMRRI SPM.....	
NTRC	HSDLDAAVSA .YQQGAFDYL PKPFDIDEAV ALVERAISHY QEQQQPRNIE	
SPOOF	YGELDMIQES .KELGALTHF AKPFDIDEIR DAVKKYLPLK SN.....	
CHEY	EAKKENIIAA .AQAGASGYV VKPFTPATLE EKLNKIFEKL GM.....	
ETR	GNTDKSTKEK CMSFGLDGVL LKPVSLDNIR DVLSDLL...	
DZ2	151
OMPR	OmpR = <i>E. coli</i> (Involved in osmoregulation)
PHOB	PhoB = <i>E. coli</i> (Involved in phosphate utilisation)
NTRC	VNGP	NtrC = <i>S. typhimurium</i> (Involved in nitrogen utilisation)
SPOOF	SpoOF = <i>B. subtilis</i> (Involved in sporulation)
CHEY	CheY = <i>E. coli</i> (Involved in chemotaxis)
ETR	ETR = <i>A. thaliana</i> ETR1 gene encoding an ethylene receptor (partial amino acid sequence)

The predicted protein sequence of DZ2 is shown in bold as are the conserved amino acid residues required for phosphorylation of the protein

FIG. 2

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Expression analysis of DZ2 in various plant organs using Northerns

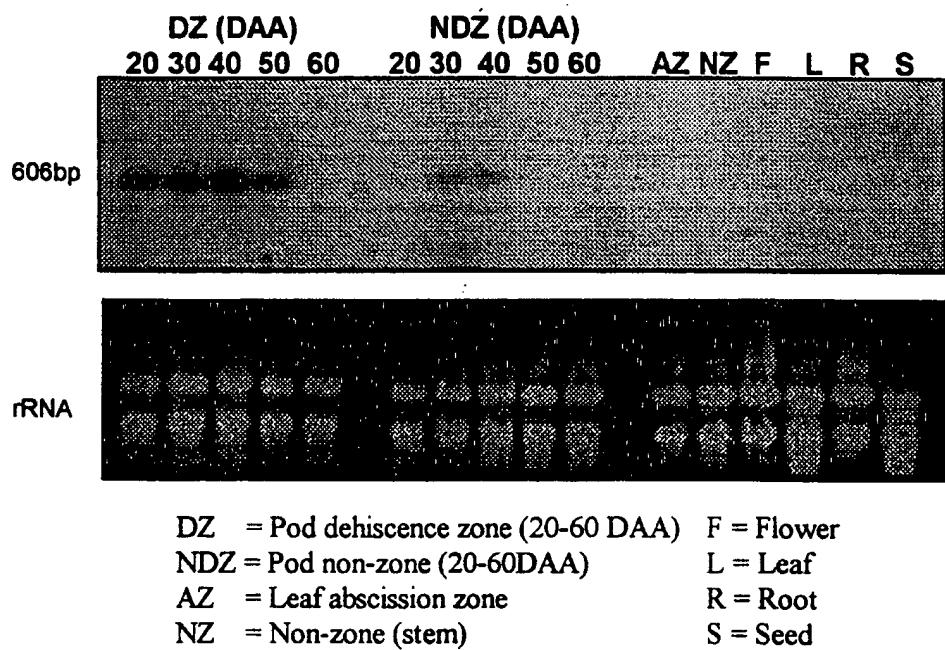


FIG. 3

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Comparison of bacterial two-component regulatory systems with DZ2

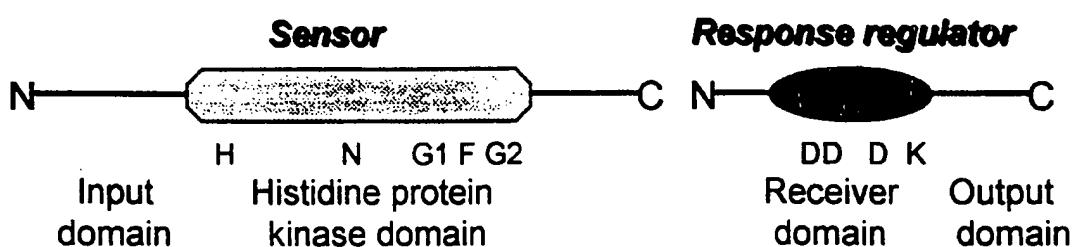
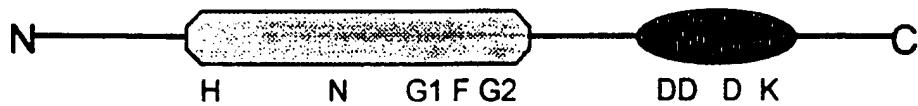
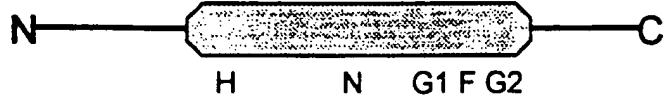
Bacterial two-component system***Arabidopsis thaliana ETR1 gene******Arabidopsis thaliana ERS gene*****DZ2**

FIG. 4

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FIG. 5

TATATAAATTACGGTTAACAGATAATGTTCTGGTTAAATTGTAATTCTNATGTGCCNNTCAAANTTTTATTNTNATTNGT	78
TTACTAGGGACATTAAGTAAACNTTTATATCATGTAACAAAAAAAACNTTTTATATNTCAACTATGA	156
CGAATTATTCTTAGTGTTCAGAAATTGACGACAAACCTAACCTAACAAATTAAATTGACGTTAGTT	234
AAGTAATTATTATAAGTGGATAAAATTGAGCAAGCACATTACGAACACTGGGATCAAGGAGAGTCACAATTAAATTCTTA	312
GGCTCTAGACGAAC TGCGGGAGCAAG -> DZ2BGENF	
Xba.I	
CGTTATACACAAATTATCTAAATACTATATATACAGCTGCATGCTACCGATAATGATCAAATGTTTATGTACTT	390
TTCAAGC GAAAATTCTTGTGCCATACATTACTGTGTTAATGAAATCATTAAATATGTGAAGCCAGGAAAAGAGTACAAAA	468
GGAGTTTGTGAGGCATTTCGGAGACACTGAATGTGAATAATAAAAGGAATTGCCGAATTGATTCTAGTTGGT	546
GAAAGTGGCTGAAAATTGTATGTCCATTGCTTATAAAACTATAAAATAATTNTNATATTACTNTGGACATTAG	624
TINNGATAGACCCCTAGCTAAAMTTTTAAAAATTATACATTCAATTCTNAAGTACCAAACTTAATATCACAAATCGGA	702
TAAAATTGCTTAAGAAACCATTACAAACTCAGCTTGTGGACTCTGAGAGAAAACTAAGAGCTAGACATAACGGTTAGTAG	780
TGTAGCCGCATTTTATGCTTAATTGCTTAAGCATGACTCTATGCTCCTTGATGATATTNTTAATATCCTAG	858
GACATATGGATTGATAAAGATCTTATCACACGACATTAGCTCAACAAACAAAATACTGAAAGTATAT	936

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AATCTGGTACAGAAATTCTTATGCCAAAATACTATAATTAGAATTTCGTTATGATTAAGATGAATTATTAA 1013

ATTAATATATTTCACCTTTGTTTCTTATGTATTCTTAGTATTGTTACCCATTATTGACCGATTGGTGTCAATTAA 1092

GTTGGTAAGACAACACTCAGTGCACGATGCAGATTACATTCAAGGAAGATTCACTGTAAGAAAGATATTTCGCTTGT 1170

GGTGTGAAAATATGCCTCTTCACTTTCAACTATAAAATTTCGATCGATGTATCTACGTTAACACAATTCAAC 1248

AATCTTCTTCTAGAATCCA AAAATTGTAAGCCGGTTCTAATCTCTTCAGTATACTATGTAATATGTATGCATATA 1326

TTATTATTCTATAACAAACAGAACCCATGCATGCAAGAAGATACTGTTACACGGCTCATAACAAACACAAAAACATA 1404

CGCATGCCATTAGAACACTTGTATGTTAATTTCATTAATGTTTGCCATAAACATTCTTCGTTTAATTAGCTTCTTTT 1482

NCOI

< - GTCGGAAAGAAAAAACCGACCATTGGCAG DZ2BGENR AACCAAG
GTGTGAAGATTGTTCGAAGAAAAAACCGAACGATGGCAACACATCCACGGAGATACTCAAGAAAACCAAG 1560
M A T T S T S T G D I K K T K

TCACTGAAACTG -> DZ2BFL

TCACTGAAACTGAAAGAAACTTAACGTGTTGATGATGATACTGTAATTTCGTAACCTTCACGGAGATAATTC 1638
S V E V K K L N V L I V D D T V I R K L H E N I

ATCAAATCGATCGGTGGAATTTCACAGACGGCTAAGAACGGTGAACATCCACCGGACGGCAATGCA 1716
I K S I G G I S Q T A K N G E E A V N I H R D G N A ->

FIG. 5 CONT'D

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A) D₂Z₂B cDNA sequence (top) -
D₂Z₂ sequence (bottom).

FIG. 6

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FIG. 6 CONT'D

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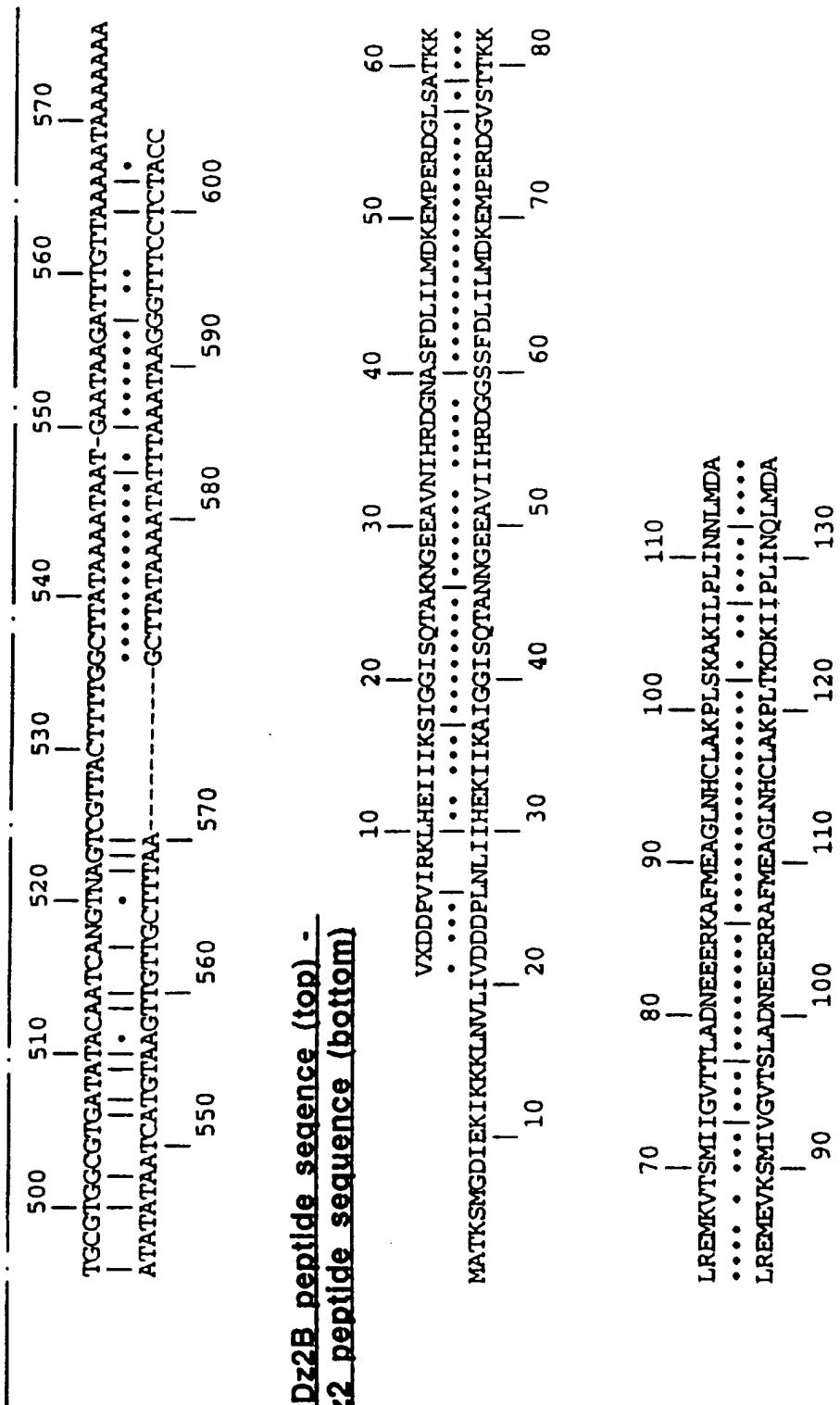
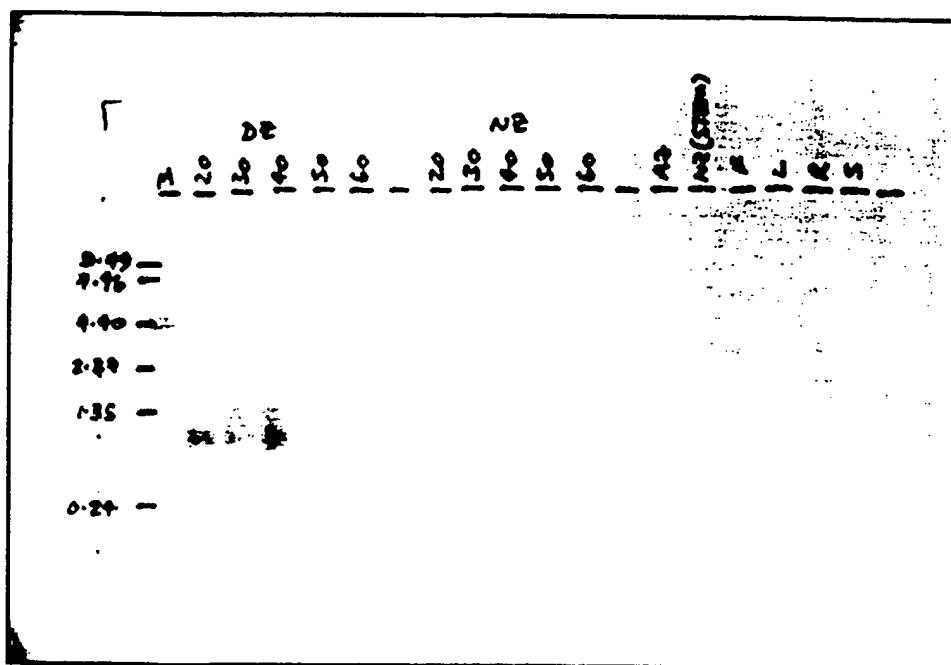


FIG. 6 CONT'D

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FIG. 7
NORTHERN ANALYSIS OF EXPRESSION OF
DZ2B IN PODS AND OTHER TISSUES



DZ = POD DEHISCENCE ZONE (20 - 06 daa)

NZ = POD NON-ZONE

AZ = ABSCISSION ZONE

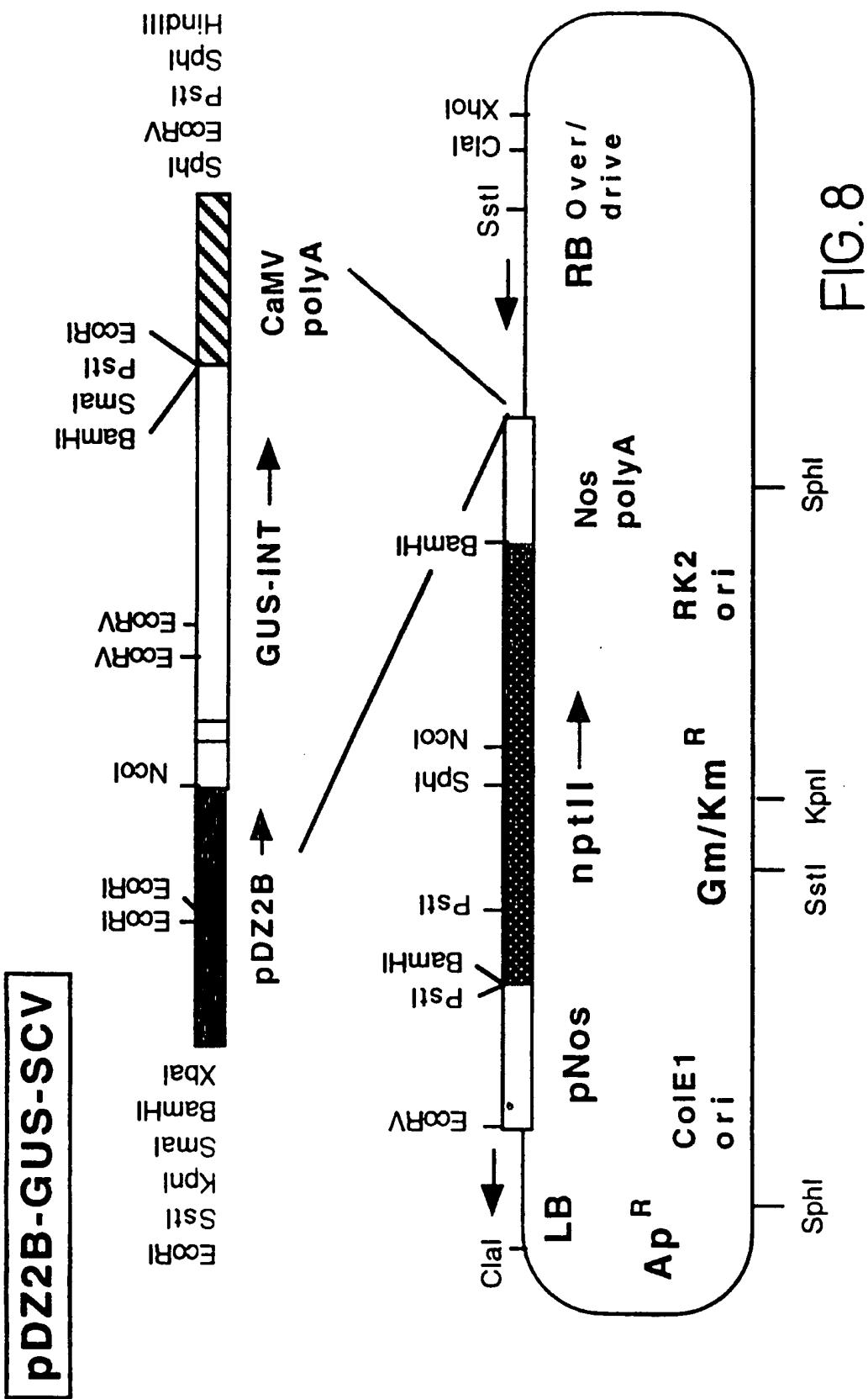
F = FLOWER

L = LEAF

R = ROOT

S = SEED

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FIG. 9

ATATATGTGATAACAGATAACATCTTATACAAATTAAACACCGAAACCATACTACATGCACGGTGTGATCACACACGGCACACA 78

CATAGAACATACACCGAATAATTTCCTTACAGTTTAATTTCATTTCATTAACTTACTCTTTTTGGTGAAGAT 156

TCTTGAGAGAAAATCGAACAAATCCACCGGAGGTACCGAGAAAACCAAGTCGATAGAAGTGAAGA 234
M A T K S T G G T E K T K S I E V K K 13/27

<- CGATCG
AGAAACTAATCAACGTGTGATCGTGTGATGATCCATTAAACCGTAGACTCCACGAGATGATCATCAAAACGATCG 312
K L I N V L I V D D P L N R R L H E M I I K T I G

<- CGAATAACGGTGAGGAGGCATTAAATCA GW1

GAGGAATTCTCAGACTGCAA AT3GW2
GAGGAATTCTCAGACTGCAAAGAATGGCGAAGAGGGCNGTGATCCTCACCGTAGCGGGAAGGATCTTCGACCTTA 390
G I S Q T A K N G E E X V I L H R D G E A S F D L I

TTCTAATGGATAAGGAAATTGCCTGAGAGGGATGGCAGTTCGACCAATTAAAGANGCTTAAGAGAAATGAAAGGGGACGTCAA 468
 L M D K E M P E R D G V S T I K X L R E M K G T S M

TGATCGTTGGGTAACGTCAAGTAGCTTGACCAAGAAGAGCCGTAAGGCTTTATGGAAGCTGGCTCAACCATTGCT 546
 I V G V T S V A D Q E E R K A F M E A G L N H C L

TGGAAAAACCCCTAACCAAGGCAAGATCTTCCCGCTCATTAGGCCACCTCTTCGATGGCTGATGGATGAAGGCTCATT 624 14/27
 E K P L T K A K I F P L I S H L F D A .

AATGATCTATATTTCATGAAATCACCTAACACGTGATTGACACAAAATCTGCATTGTGATATAAGGG 702

TTTCTCATATGTTGATTATTCTTATCGTCGGAGGTAAATCATGCAAGTCATTCTTTGGCTAATAAAA 780

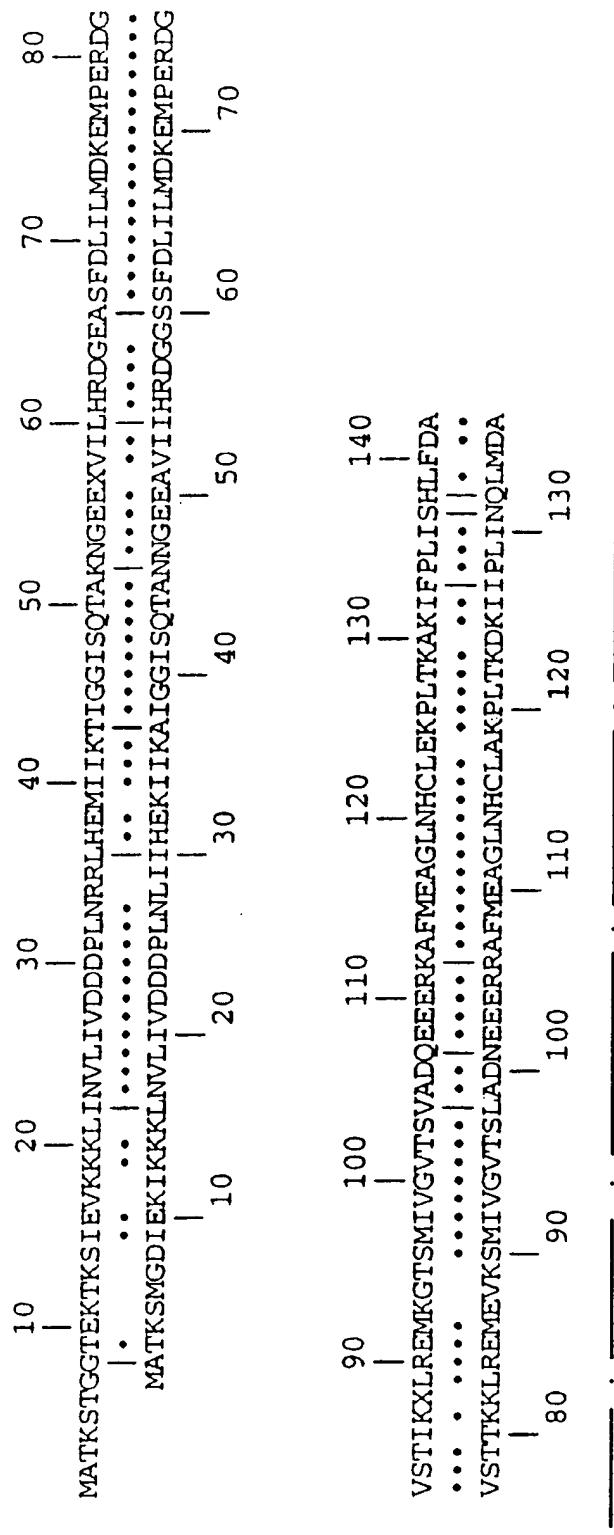
TATTAATAAGGTTTCTCAAAAAAAAAAA 818

FIG. 9 CONT'D

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FIG. 10

A) DZ2AT3 peptide sequence (top)
DZ2 peptide sequence (bottom)



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B) DZ2A13 peptide sequence (top)
DZ2B peptide sequence (bottom)

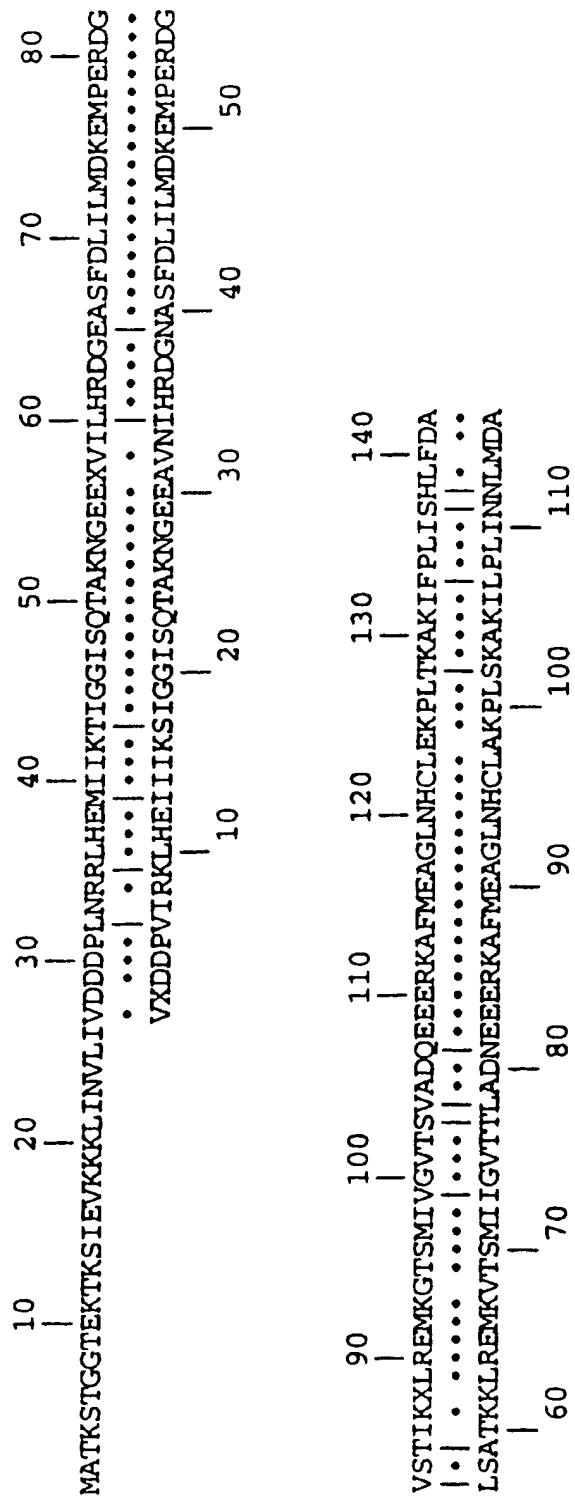


FIG. 10 cont'd

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FIG. 11

GTAATGGCACTATAAGGCACGGTGGTCGACGGCCGGCTCGTATTGGCCCATGGCTACT	78
<u>CACTACTAGGGCACGGTGGTC</u> -> ATDZ2F	
SpeI	
AAAACAGTTCACGATTGTCCCCCTTAATTAAACATGTATGGGATATTGGCTATAAATTATG	156
TAAAAAATTTCACGGATAGATTGTGAATTTCGAGTTAAATATCTTCAAATTACCTCACATTACAAAAA	234
GGTAGAACCTGTTGAAAAACTTAATGCTCTATAAAACACTAGACAATAACAAAATACGTAATGGCTAAAGAACCTAAATT	312
ATGATTTTATTATCTTCCCTTCCGTGAGTATAAGCCATTTCATAGTAAAAGCATTACGAATAAGACATTG	390
AACACTACTGACATATAAGTAGATTGATGGGTTAACTTGATGGCTTAAGCATGAACTTCAATG	468
CTTTTATAAAACTTCACTGAGAATTATTCCCTCGTTCTATACTAGCAGAAGGGTTCGATAGTGATTTACAACCGTTCA	546
AAACAAAAACCTTTAACCCAAAAACCAAGAAATGAAGNTATCTAAACTGATTATACATTCTGTCTAAATTATCAAA	624
ATAACATACTCTCTTGTTTACTTATAAACGATATGAAAGAACATAGAATCTTATTATGATCT	702

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AGAAGAAATTAAAGAAATATATAATTTCATTCTTCACTCATGGTTCTTACATTCTTAATTGTT 780
CACCATTGATTACTTGTCTCATATTAGTTGTACTACAACCTGAGATAATGATTACATTTCAGCCAA 858
ATTCATGTAAGAGATGCTTTCTTGTGATGTTAAATGCTTCTTCACTTTTCTTAACTATAAT 936
CTTGATCGAATGCCAACCTCTTAGAACATAAGATCTTAAATCCAATCGTAGGCCACTATTCATTACT 1013
TATGTAATTATATGTGATACAGATAACATNTATAACAAATTAAACACGAAACCATACATGCACGGTGTGATCACACACG 1092
CACACACATAGAAACATAAACACGGAATAATTCTTACAGTTAACCTTACTTCTTTTTGTT 1170

NcoI
<- CTTGAGAGAAAGAAATCGACCATGGAA
GAAGATTCTTGTGAGAGAAAGAAATCGAAGATGGCAACAAAATCCACCGGAGGTACCGAGAAACCAAGTCCGATAGAAG 1248
M A T K S T G G T E K T K S I E V

TGAAGAAGAAACTAATCAACCGTGTGATCGTGTGATGACTCCACGGAGTCACTCAA
K K K L I N V L I V D D P L N R R L H E C H Q -> 1324

FIG. 11 CONT'D

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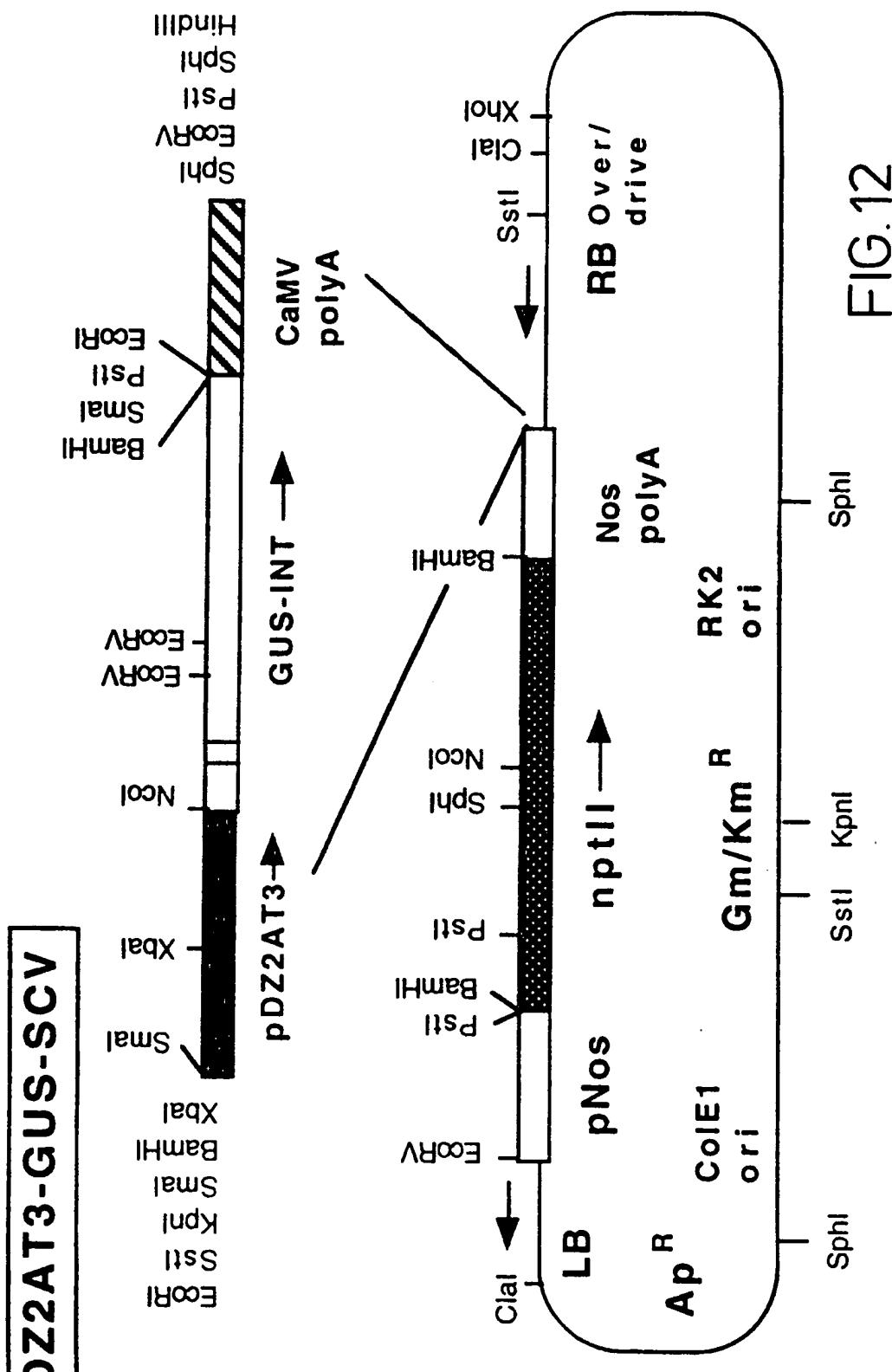


FIG. 12

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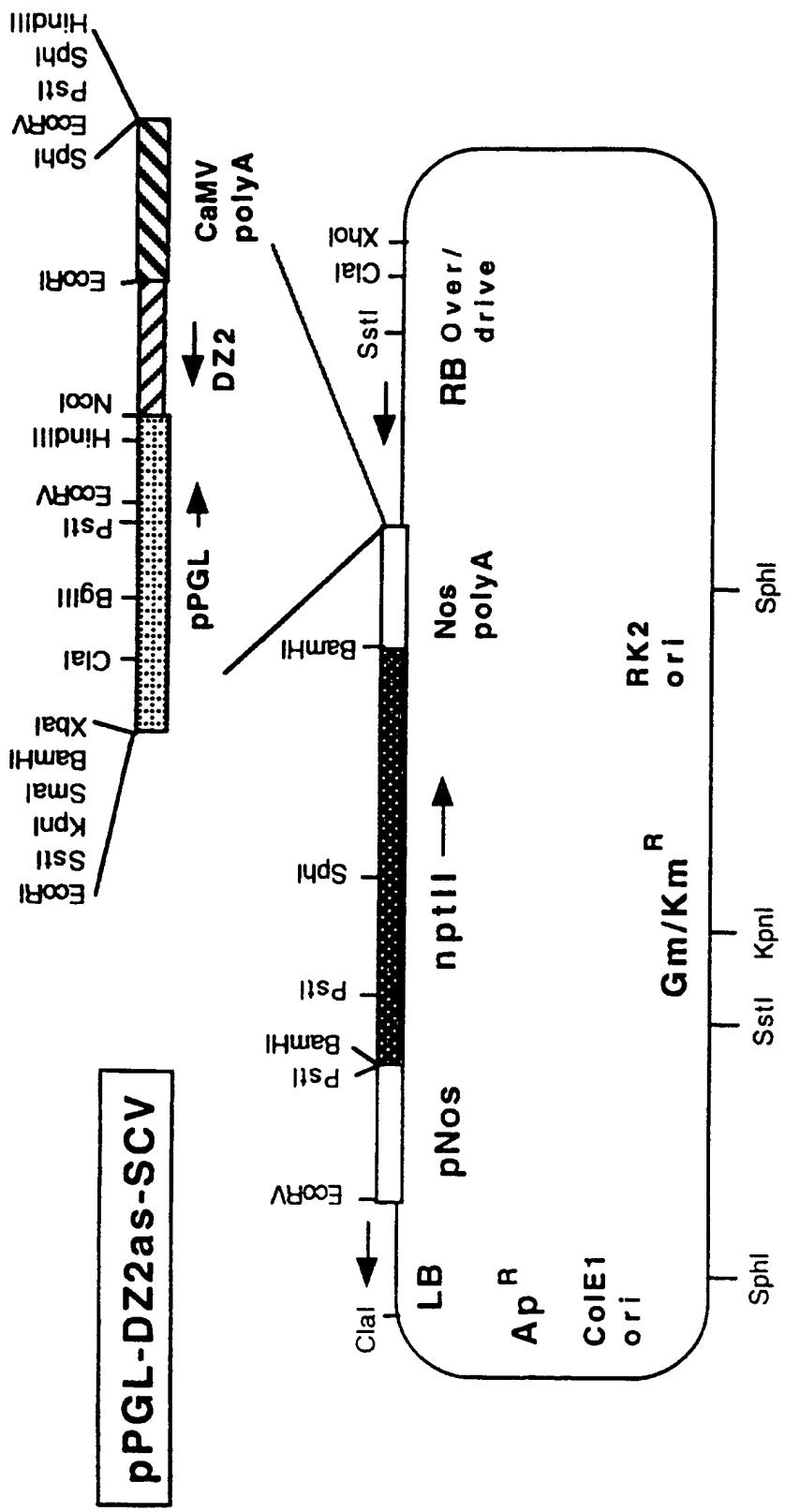


FIG. 13A

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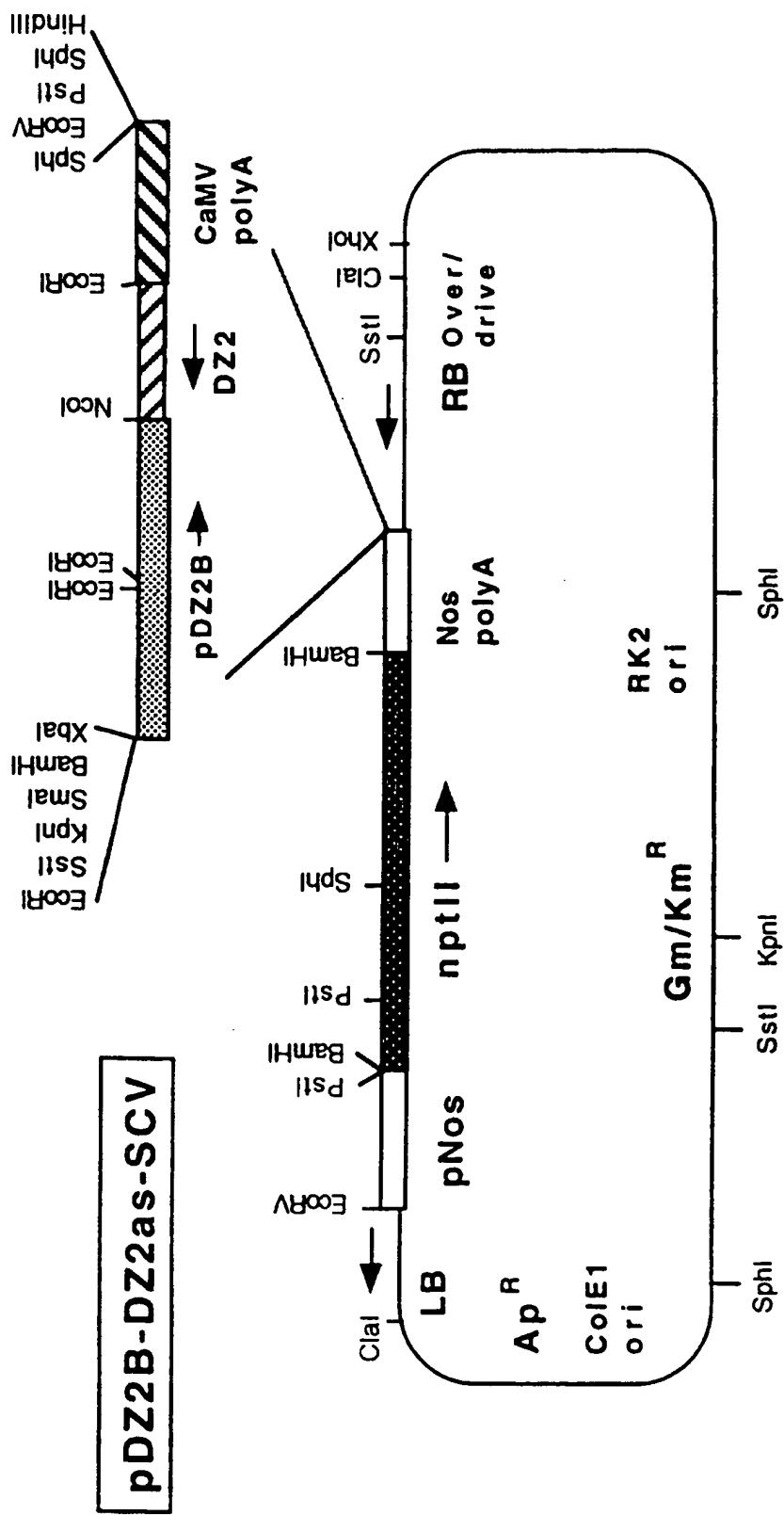


FIG. 13B

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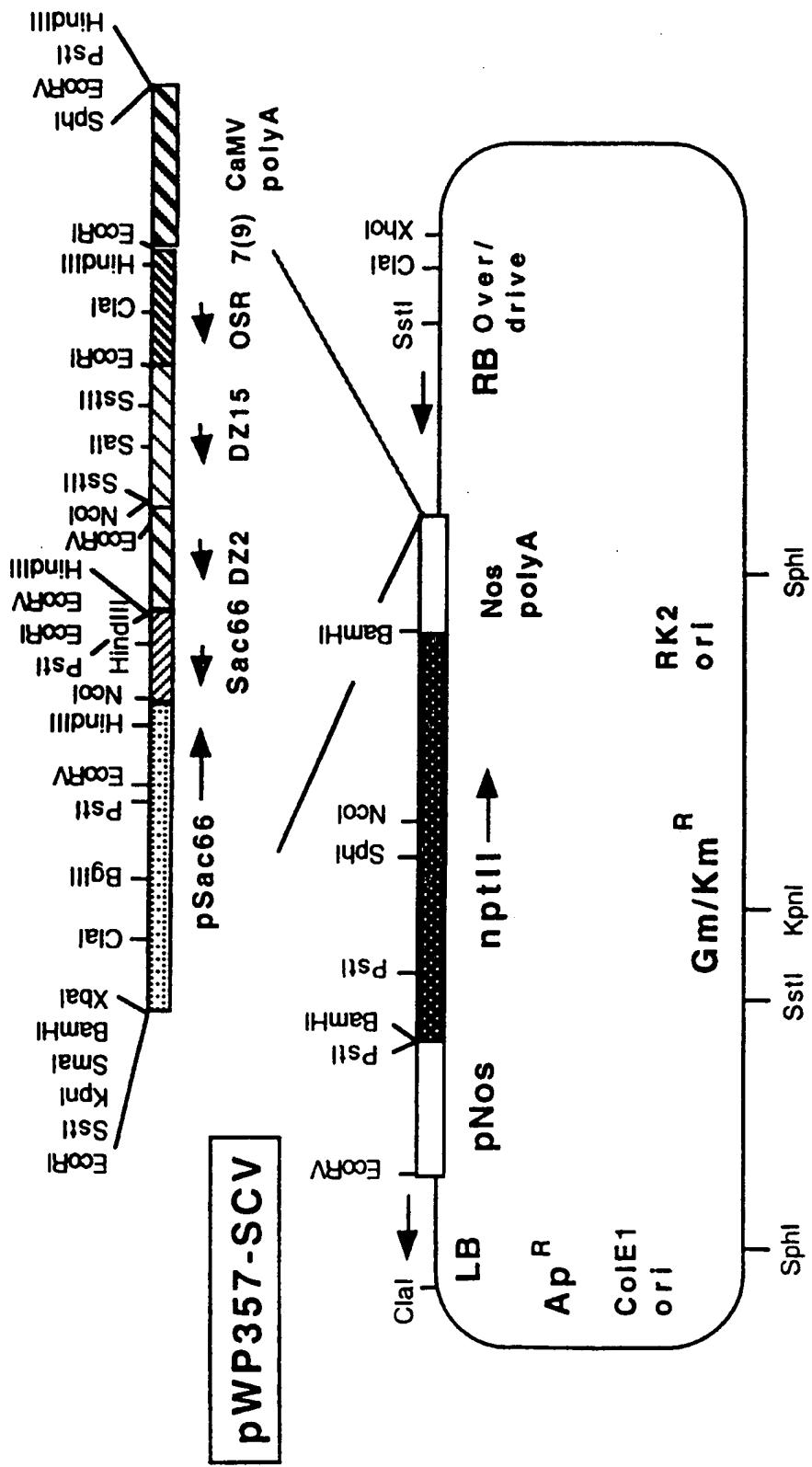


FIG. 14

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FIG. 15

GGCATCACGGTACCCGTAATCCACCATACAAACAAAGTTCTGTGAAAGTCTCCCCA 60
AAACTGCCAACAGACTCATATTAGTTACTCTAGAAATAAACACAGTGTTCCTGAA 120
AAGATTAGC GTTCAACCCGAAATGCCCGTTGTCATGGAAGTCTGCCTATTCTTA 180
M A R C H G S L A I F L 12
TGC GTTCTTGATGCTCGCTTGCTGCCAACGCTTAGCAACGTAGATGGATAT 240
C V L L M L A C C Q A L S S N V D D G Y 32
GGTCATGGAAGATGGAAGCTTCGAAACCGATAGTTAATCAAGCTCAAACGAC 300
G H E D G S F E T D S L I K L N N D D 52
GTTCTTACCTTGAAAAGCTCCGATAGAACCCACTACCGAATCATCAACTGTTAGTGT 360
V L T L K S S D R P T T E S S T V S V S 72
AACTTCGGAGCAAAAGGTGATGGAAAACCGATACTCAGGCTTCAAGAAAGCATGG 420
N F G A K G D G K T D T Q A F K K A W 92
AAGAAAGGCATGTTCAACAAATGGAGTGACTACTTCTGATTCTAAAGGAAAGACTTAT 480
K K A C S T N G V T T F L I P K G K T Y 112
CTCCCTTAAGTCTTACGAGGCCATGCAAATCATTAACGTAGCTTCCAGATCCTA 540
L L K S I R F R G P C K S L R S F Q I L 132
GGCACTTTATCAGCTTCTACAAAACGATGGGATTACAGTAATGACAAGAACCTGGCTT 600
G T L S A S T K R . S D Y S N D K N H W L 152

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ATTTGGAGGACGTAAATAATCTATCAATCGATGGGGCTCGGGGGATTCGGATGGC 660
I L E D V N N L S I D G G S A G I V D G 172
AACGGAAAAATCTGGTGC~~AA~~ACTCATGCCAAATCGACAAATCTAAGCCATGCCACAAA 720
N G K I W W Q N S C K I D K S K P C T K 192
GCCCAACGGCTCTTACTCTACAACCTAAACAATTGAAATGTGAAGAGAGTG 780
A P T A L T L Y N L N N L N V K N L R V 212
AGAAAATGCCACAGCAGATTCAGATTGAGAAATGCCAACAGTGTGATGTTAAGAAT 840
R N A Q Q I Q I S I E K C N S V D V K N 232
GTTAAGATCACTGCTCCTGGCATA~~G~~TCCCACACGGATGGTATT~~C~~ATATCGTTGCTACT 900
V K I T A P G D S P N T D G I H I V A T 252
AAAACATT~~C~~GAATCTCCAATT~~C~~AGACAT~~T~~GGACAGGTGATGCATATCCATTGAG 960
K N I R I S N S D I G T G D D C I S I E 272
GATGGATCGCAAATGTTCAAATCAATGATTAACTTGGCCCCGGTCA~~T~~GGCATCAGC 1020
D G S Q N V Q I N D L T C G P G H G I S 292
AT~~T~~GGAAACCTTGGGGATGACAATTCCAAGCTTATGTTATCGGGAAATAATGTGGATGGT 1080
I G S L G D N S K A Y V S G I N V D G 312

FIG. 15 CONT'D

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GCTTACGCTCTGAGACTGACAATGGAGTAAGAACATTCAAGACITTAACCAAGGGAGGGTCAGGA 1140
 A T L S E T D N G V R I K T Y Q G G S G 332

ACTGCTAAAGAACATTAATTCCAAAACATTCGTATGGATAATGTCAGAACATCCGATCATA 1200
 T A K N I K F Q N I R M D N V K N P I I 352

ATCGACCAGAACACTACTGGACAAAGGACAAATGCGAACAAACAAGAACATCTGGCTCAAGTG 1260
 I D Q N Y C D K C E Q Q E S A V Q V 372

AACAATGTCGTCTATCGGAACACATAACGGTACAGAGCGCAACGGATGTGGCGATAATGTT 1320
 N N V V Y R N I Q G T S A T D V A I M F 392

AATTGCAGTGTGAAATATCCATGCCAAGGTATGTGCTTGAGAACATCAAAGGA 1380
 N C S V K Y P C Q G I V L E N V N I K G 412

GGAAAAAGCTTCTGCAAAAATGTCAAATGTTAAGGATAAAGGCACCGTTCTCCTAAATGC 1440
 G K A S C K N V N V K D K G T V S P K C 432

CCTTAATTACTAAGTTGATTATGTAATACATAAAATACGTATTATATGTGGTTATAGAT 1500
 P P 433

GCCATCTATATCCTTATCTCGATATATAGAAAACTAAGGATTAT 1560

GGGAATATACAAATAGTTGAGATAATTGTTGCTGTATATGGTTCACTGAAGTTGA 1620

TTGCTTGTCCACCGAATAATGAAATAATGTCATTGTC 1657

FIG. 15 CONT'D

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aggtgaccgttgctgatggcaatgtgctggtaagcgagaggtagacggtgccggata
V T V A D G N V L V K R E V D G G L E T
cagttaaagtcaaattgccagctgtcattagcgccgacttgcggctcaatgagccgcgg
V K V K L P A V I S A D L R L N E P R Y
acgctactctgcccaatatcatgaaggccaagaagaagccataaaaagctcacagcca
A T L P N I M K A K K K P I K K L T A T
cagatgtcggtgtggacttggcgccacgtcaacaagtgtttagcgtagaagacccgccc
D V G V D L A P R Q Q V L S V E D P P T
ccagacaggctggccattgtgcctgatgtcgacactctcatcaccaagttgaaagaaa
R Q A G S I V P D V D T L I T K L K E K
agggtcattttaatgcaatgtcaccaatacagtttttagtttacaaatttttcgt
G H L *
gagggtttcagctgttaccaataatatttttcaaaatcgattttttacttgcattt
taaaagatcaaataatataatacaatgaacatttttaacagcaatcttttttatattt
tggagatttcatcgacttatgtcataatttttatcaatttattgtttagtttagtgcattt
atataataaaagtatgtttctggtaaaaa

FIG. 16

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OSR79 306bp

5' . ggttgggtcgaaaccataagggtggaaaggccttttctcgccttgacaaatccctctgggt
 L G R T I G G K L L S L D K S S G S .
 . cgggttttcagttcccatcaggaggcttatggtaaaagctgagggttcaaattgaaacttgc
 G F O S H Q E F L Y G K A E V O M K L V .
 . tccctggtaactctgctggaaacacagtcaacaattttatccatccatccatccatccatca
 P G N S A G T V T F Y L K S P G T T W .
 . ggatgagatcgatttcgagttctggggaaacataaggccatccatccatccatccatca
 D E I D F E F L G N I S G H P Y T L H T .
 . ctaatgtttacacacgaaaggctctggagagacaacaggcagttcatctatgggttcgac
 N V Y T R R Q R T A V S M S V R P .
 . ccgacc 3'
 D

FIG. 17